

# Topological interactions in DNA catenanes

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The elasticity of DNA catenanes, i.e. multiply linked DNA rings, is investigated using the Gauss invariant as a minimal model for topology conservation. An effective elastic free energy as a function of the distance  $R$  between segments located on different rings is obtained. An anharmonic part at large distances, growing as  $R^4$ , if  $R \gg R_G$  ( $R_G$  being the radius of gyration of a random walk ring) is found, while for  $R \ll R_G$  the interaction is strongly repulsive. Treating the attractive interaction as the dominant one, distribution functions for the distance between segments located on different rings for several linking numbers are derived which are in qualitative agreement with distributions functions obtained experimentally from electron micrographs of DNA catenanes (S. D. Levene et al., *Biophys.J.* **69**, 277, 1995).

Knots and links made from DNA beautifully elucidate the general role of topology in nature. More specifically, DNA rings from bacteria such as *Escherichia coli* form so-called catenanes (the chemical term for entangled rings called links by knot theorists) as intermediate products of DNA replication and recombination [1]. Together with single knotted DNA rings they form the class of so-called topoisomers, i.e. topologically distinct isomers. They can be isolated experimentally by manipulating certain enzymes (topoisomerases) switching back between various topologies [1,2]. Several catenanes and knots have been identified e.g. by electron microscopy and electrophoresis [1,3]. Most recently, the conformation of open-circular, multiply linked dimeric DNA catenanes has been studied by electron microscopy [4]. From the tracing of the molecular contours on the micrographs, in particular, distribution functions for the distance (in the projection plane) between segments on separate rings have been established. As the linking number - measuring the degree of concatenation between pairs of rings - is increased, the catenane conformation becomes more and more compact due to an effective attractive interaction - of topological origin - between segments.

In this letter, we present a theory to explain the experimental results of ref. [4] mentioned above. From a theoretical point of view, the statistical mechanics of entangled rings is a generally unsolved problem [5,6]. The essential difficulty is how to specify the topological state of the system, which is assumed to remain unchanged with respect to conformational fluctuations. The mathematical answer to this question is a so-called link invariant, various types of which are discussed in knot theory while none of them is one-to-one [7,8]. The most simple, yet most crude invariant is the Gauss integral for two given closed loops  $\alpha$  and  $\beta$

$$\Phi(C_\alpha, C_\beta) \equiv \Phi_{\alpha\beta} = \frac{1}{4\pi} \oint_{C_\alpha} ds \oint_{C_\beta} ds' \dot{\mathbf{r}}^\alpha(s) \wedge \dot{\mathbf{r}}^\beta(s') \cdot \frac{\mathbf{r}^\alpha(s) - \mathbf{r}^\beta(s')}{|\mathbf{r}^\alpha(s) - \mathbf{r}^\beta(s')|^3}, \quad (1)$$

which is a double line integral over the loop contours  $C_\alpha, C_\beta$  where  $\mathbf{r}^\alpha(s)$  denotes the segment position in  $d = 3$  space dimensions as a function of the chain contour. Unfortunately, the Gauss invariant has the same value for two unlinked rings and the so-called Whitehead link, and is therefore not one-to-one. In fact a whole series of higher-order linking coefficients (HOLC) needs to be considered in order to uniquely characterize the link topology. A different type of invariants, known as knot (or link) polynomials and defined via skein relations, are suitable for computer simulations [9–13], but not for an analytical theory as they are independent of the segment positions and therefore cannot be coupled to a particular polymer model. Recently however, a deep connection between the Gauss invariant as well as HOLC and polynomials for knots and links has been established [14]. As a consequence, it has been demonstrated that the Gauss invariant is a first order approximant to the true topology of links [15]. It is however no invariant for knots [16,9] as was supposed in [6,17].

In view of the experimental data, however, we have additional reasons to use the Gauss invariant more safely. The dimeric DNA catenanes are only interlinked and the individual rings are not self linked. Moreover, the Gauss invariant has proven numerically to be very efficient for simple models describing the polymer conformation, such as the random walk and the self-avoiding walk [18–20]. It was especially shown that almost 95% of the conformations could be found by using the Gauss invariant, compared to the resulting number obtained by the simulation [20].

Given these facts about the Gauss invariant, we propose to use it as a minimal model for topology conservation in polymer ring systems. In what follows we develop a framework for an arbitrary number of concatenated rings. Finally, in order to make contact with the experiments on DNA catenanes, we restrict our analysis to two concatenated rings. We will consider random walk rings with *no* additional local excluded volume interactions other than those

imposed by topology conservation. This procedure may appear strange, but it assures the rigorous applicability of the Gauss invariant in order to classify the pair-wise topology of rings as self-intersections are allowed for a single ring. This ansatz neglects excluded volume interactions of a ring with itself, but maintains effectively the excluded volume interactions between different rings as a result of topology conservation. The latter is imposed by fixing the linking number matrix  $m_{\alpha\beta} = \Phi_{\alpha\beta}$  between rings  $\alpha$  and  $\beta$ . Furthermore, we keep one point fixed on each ring, i.e.  $\mathbf{r}_0^\alpha$  for every ring  $\alpha$ . Our goal is then to calculate the partition function  $Z(m_{\alpha\beta}; \{\mathbf{r}_0^\alpha\}) = \langle \delta(m_{\alpha\beta} - \Phi_{\alpha\beta}) \rangle_{\{\mathbf{r}_0^\alpha\}}$ , taking the average over random walk ring conformations with the given constraints. The use of a random walk as a conformational model for DNA rings is of course insufficient as the local bending stiffness is neglected. It has been shown however, that for large chain lengths, DNA rings without supercoils behave like Gaussian rings [21] which corresponds to the experiment in [4] discussed at the end of this letter.

We now present the general route to calculate  $Z(m_{\alpha\beta}; \{\mathbf{r}_0^\alpha\})$  (the details will be presented elsewhere). The two ring partition function has to be computed subject to the constraint of the topology conservation. The latter is expressed by a simple delta function constraint, which maintains the linking number  $m_{\alpha\beta}$  between the two rings, using the Gaussian invariant. A Hubbard - Stratonovich transformation handles the Gaussian integral and introduces a conjugated field theory. The partition function is then Fourier transformed to  $Z(s_{\alpha\beta}; \{\mathbf{r}_0^\alpha\})$  where  $s_{\alpha\beta}$  is the symmetric part of the matrix conjugate to  $m_{\alpha\beta}$  (the general approach to define the problem for many-rings systems is out-lined in detail and solved for  $m_{\alpha\beta} = 0$  in ref. [22]). In the conjugate partition function  $Z(s_{\alpha\beta}; \{\mathbf{r}_0^\alpha\})$ , the Gauss invariant appears as an effective (imaginary) interaction which can be re-expressed using collective variables, i.e. the tangent vector density of ring  $\alpha$ ,  $\mathbf{j}^\alpha(\mathbf{x}) = \oint ds \dot{\mathbf{r}}^\alpha(s) \delta(\mathbf{x} - \mathbf{r}^\alpha(s))$ .

We then use the fact that a symmetric matrix  $s_{\alpha\beta}$  can be decomposed according to  $s_{\alpha\beta} = \sum_\gamma \eta_{\alpha\gamma} \eta_{\gamma\beta}$ , and define a modified tangent vector density according to  $\Psi^\alpha = \sum_\beta \eta_{\alpha\beta} \mathbf{j}^\beta$ . The variables  $\Psi^\alpha(\mathbf{x})$  are introduced into the partition function  $Z(s_{\alpha\beta}; \{\mathbf{r}_0^\alpha\})$  using conjugate gauge fields  $\mathbf{B}^\alpha(\mathbf{x})$ . The modified tangent vector densities  $\Psi^\alpha$  are finally summed over to give the following conjugate partition function:

$$Z(s_{\alpha\beta}; \{\mathbf{r}_0^\alpha\}) = \int \prod_\alpha \prod_{\mathbf{k} \neq 0} d\mathbf{B}^\alpha(\mathbf{k}) \exp \left( - \sum_\alpha \int_{\mathbf{q}} B_\mu^\alpha(\mathbf{q}) B_\nu^\alpha(-\mathbf{q}) \epsilon_{\mu\lambda\nu} q_\lambda \right) \times \left\langle \exp \left( -i \sum_\alpha \int_{\mathbf{q}} \hat{\Psi}^\alpha(\mathbf{q}) \cdot \mathbf{B}^\alpha(-\mathbf{q}) \right) \right\rangle_{\{\mathbf{r}_0^\alpha\}} \quad (2)$$

Here  $\epsilon_{\mu\lambda\nu}$  is the standard Levi - Cevita symbol. Thus, the linking number constraint has been rewritten in terms of an  $n_p$ -component Abelian Chern-Simons field theory. In the case of DNA catenanes,  $n_p = 2$ . The next step is to perform the average over the random walk rings  $\{\mathbf{r}^\alpha(s)\}$  while keeping fixed the points  $\mathbf{r}_0^\alpha$  on each ring  $\alpha$ . To simplify the functional integration, a preaveraging procedure is carried out for Fourier transformed tangent vector densities  $\mathbf{j}^\alpha(\mathbf{q})$ :

$$j_\mu^\alpha(\mathbf{q}) \simeq \oint ds \dot{r}_\mu^\alpha(s) e^{-i\mathbf{q} \cdot \mathbf{r}_0^\alpha} \langle e^{-i\mathbf{q} \cdot (\mathbf{r}^\alpha(s) - \mathbf{r}_0^\alpha)} \rangle = \oint ds \dot{r}_\mu^\alpha(s) e^{-i\mathbf{q} \cdot \mathbf{r}_0^\alpha} e^{-\frac{t^2}{2d} q^2 s} \quad (3)$$

The preaveraging approximation is not critical in the physical problem discussed here. The topology is conserved and the main physical contribution is taken already by the average. The integration over the gauge fields in Eq.(2) is approximated by a cumulant expansion (as a power series in  $s_{\alpha\beta}$ ), keeping terms up to  $O(s_{\alpha\beta}^2)$ . Finally, we obtain the partition function for fixed linking number  $Z(m_{\alpha\beta}; \{\mathbf{r}_0^\alpha\})$  (after integrating over  $s_{\alpha\beta}$ ).

The resulting effective free energy for two interlinked rings  $\alpha$  and  $\beta$  (to order  $O(m_{\alpha\beta}^2)$  in the linking number) reads:

$$\beta F_{\alpha\beta}(R = |\mathbf{r}_0^\alpha - \mathbf{r}_0^\beta|) = \frac{m_{\alpha\beta}^2}{4} (2\pi)^3 \left( \sum_{j=1}^{\infty} \frac{(-1)^j}{(j-1)!(2j+1)} \left( \frac{R}{2\sqrt{2}R_G} \right)^{2j-1} \right)^{-2} \quad (4)$$

The sum can be evaluated exactly by re-summation and transformation to an integral over an exponential function. This yields an expression which contains an Error function. To compare our result to the experimental situation, seen in electron micrographs we need to project the three dimensional result of the corresponding two dimensional case. Therefore we take the asymptotics of the free energy for large and small values of  $R$ . Introducing the scaling variable  $x = R/R_G$ , Eq.(4) is approximated by a simple asymptotic form (see also FIG.1).

$$\beta F_{\alpha\beta}(x) = \frac{m_{\alpha\beta}^2}{4} \left( (2\pi)^3 \frac{32}{x^2} + 2\pi^2 x^4 \right) \quad (5)$$

The second term in Eq.(5) is asymptotically exact for  $x \gg 1$  and produces an anharmonic term on distances  $R \gg R_G$ . The result can be intuitively understood. The two linked rings must - upon relative deformation with respect to each other produce a stronger elasticity than a Gaussian chain.

To understand the behavior of the topological free energy let us come back to simple tube arguments for entangled polymers. Although the rings are confined strongly by entanglements the elasticity is purely governed by entropy. Thus let us estimate the number of configurations of the two rings when  $m$  is large and the two rings are linked many times. In terms of a tube like model the distance between two links form a primitive path step [23,24]. The mean square primitive path step length is simply given by the mean distance between two entanglements, i.e.,  $a^2 = l^2 N/m$ . The total number of constraints is the linking number  $m$  themselves. Both yield a modulus that is proportional to the square of the linking number, which is reflected by the free energy calculated above. To understand the peculiar  $x^4$  behavior let us estimate the linking constraint for the two rings, eq.(1). The lowest order cumulant of relevance is revealed by  $\langle \Phi^2(C_\alpha, C_\beta) \rangle$ , where the average is taken by the Gaussian configuration. By estimating crudely the bond vectors as unit vectors and taking into account the cross product, the average is of the order of  $N^2/R^4$ . The linking number constraint must be incooperated  $\langle \delta(m - \Phi(C_\alpha, C_\beta)) \rangle$  to the partition function. Parameterizing the delta function by an auxillary integral, and carrying out the lowest order average yields the inverse of the estimate of the second cumulant, i.e.,  $\exp(-m^2 R^4/l^4 N^2)$ , whis is calculated above. Moreover, the result  $F \propto x^4$  to this order agrees nicely with the estimation of the limits to such exponents presented by Frisch and Wasserman [25] if two linked rings are pulled against each other. Knot theory [26] suggest limits for the exponent between 2 and 4. Higher order approximations yield higher power terms, but it they can be related to the "finite extensibility" of the entangled object [24] and are of little importance here.

The first term in eq.(5) gives the repulsive interaction on small scales due to the increased probability that a segment  $\mathbf{r}_0^\alpha$  of one ring meets segments of the other one in a neighborhood close to the link. Its explicit form behaving as  $1/x^2$  is due to an approximated evaluation of an integral over  $\mathbf{k}$  space keeping only the first-order approximant. Intuitively the appearance of such a term is also not surprising. It corresponds to the confinement energy of Gaussian structures. At smaller values of  $x$  topology plays of the dominant role. Thus we expect the corresponding confinement term  $F_{\text{conf}} \propto (R_G/R)^2$  for the compression of the two entangled rings. As higher order terms are considered, the repulsive tail of the free energy Eq.(5) approaches the vertical axis with presumably a delta peak being the limiting value while the  $x^4$ -term persists. Therefore we suppose that the latter term contains the essential physics of pairs of linked rings whereas the repulsive interaction is only relevant in the case that both rings are strongly squeezed together.

We now proceed to calculate the distribution function for distances between segments located on different rings that form a DNA catenane. As suggested by Levene et al. [4], we assume that the electron micrographs of DNA catenanes display a 2-dimensional projection of the real 3D conformation. We therefore start from the following three-dimensional distribution function

$$P(x) \sim \mathcal{N} \exp(-m^2 x^4), \quad (6)$$

the variable  $x$  being a scaled distance (absorbing also the numerical factors) between two segments located on different rings, and  $m$  the linking number. Then, cylindrical coordinates  $\mathbf{r} = (z, r, \varphi)$  are used with  $x^2 = z^2 + r^2$ . Finally, the normalized 2D distribution function is obtained:

$$P(r) = C m^{3/2} r \exp\left(-\frac{m^2}{2} r^4\right) D_{-1/2}\left(\sqrt{2} m r^2\right) \quad (7)$$

The function  $D_{-1/2}(u)$  is a parabolic-cylinder function [27]. The constant  $C$  is a numerical constant subject to the constraint that  $\int_0^\infty dr P(r) = 1$ . A selection of 2D distribution functions for linking numbers  $m = 3, 5, 7, 9$  is given in FIG. 2. They corresponding curves show a remarkably good qualitative agreement with the respective distributions that are determined experimentally in [4]. Their result is reproduced in FIG. 3. The essential characteristics of this family of functions are that for increasing linking numbers, their peak values occur for lower and lower values of  $r$ . Moreover, the distribution functions become more and more narrow as  $m$  increases. This clearly indicates that catenanes of higher linking numbers show a more compact conformation. Concerning a more quantitative comparison between our theoretical work and the experimental results obtained in [4], the semiflexible nature of DNA catenanes must be taken into account [28–30]. The appropriate model is the Kratky-Porod chain [31] which - apart from the radius of gyration [32] - can be handled only in certain limiting cases [33,34], leading to new difficulties, regarding the conformational properties only. In order to keep the purely conformational part of the model as simple as possible, we confined ourselves to simple random walk rings. The latter point is legal, since the topological properties discussed above do not depend on the polymer chain model.

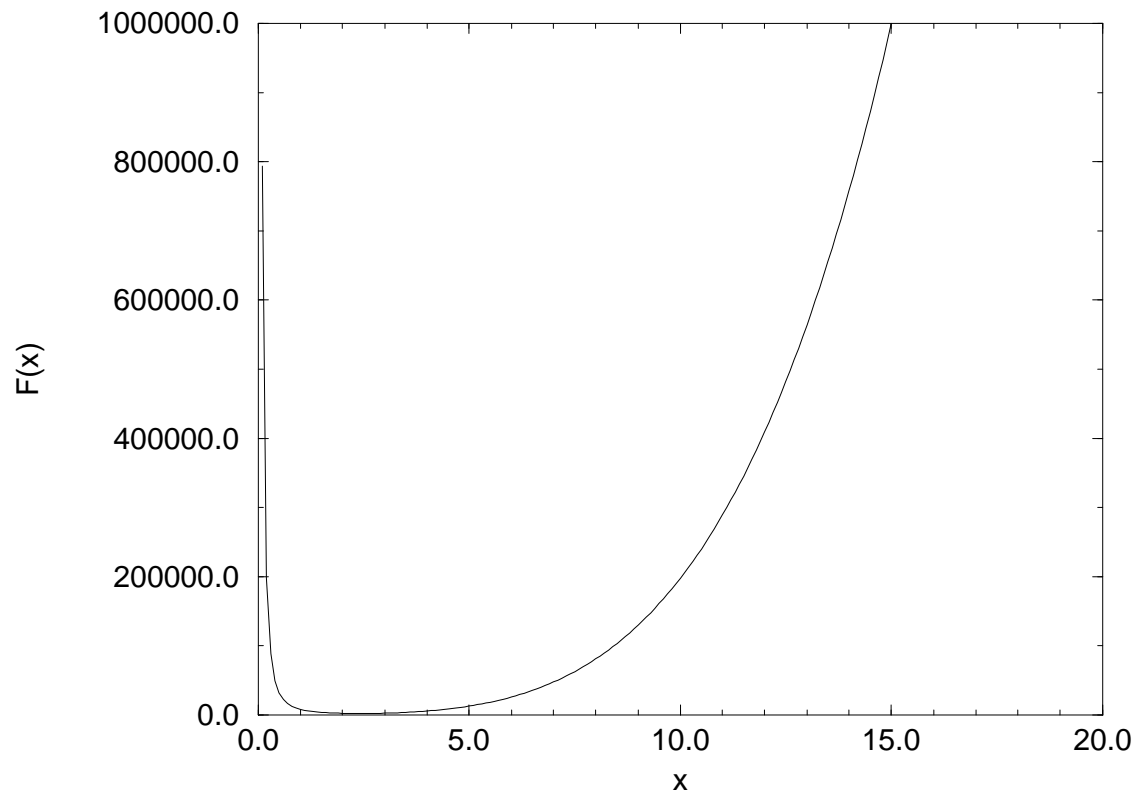
In this letter we presented a simple model for topological interactions in DNA catenanes based upon the Gauss invariant as a minimal model for topology conservation. The topological constraint of fixed linking number was expressed in terms of an  $n_p$ -component Abelian Chern-Simons field theory and was applied to the case  $n_p = 2$ . An alternative approach using Abelian BF-theory (see [35]) will be presented elsewhere. Using a pre-averaging procedure and considering the effective topological interactions to lowest order in the variable  $s_{\alpha\beta}$  (conjugate to the linking number  $m_{\alpha\beta}$  between rings  $\alpha$  and  $\beta$ ), an effective free energy was obtained for catenanes where the positions of one segment on each ring  $\mathbf{r}_0^\alpha$  and  $\mathbf{r}_0^\beta$  were kept fixed. Treating the resulting  $R^4$  term which is both attractive and strongly anharmonic as the dominant one, we obtained distribution functions for distances between segments located on different rings. The 2D projections of these distribution functions show a good qualitative agreement with recent experimental data on the conformation of DNA catenanes with different linking numbers [4].

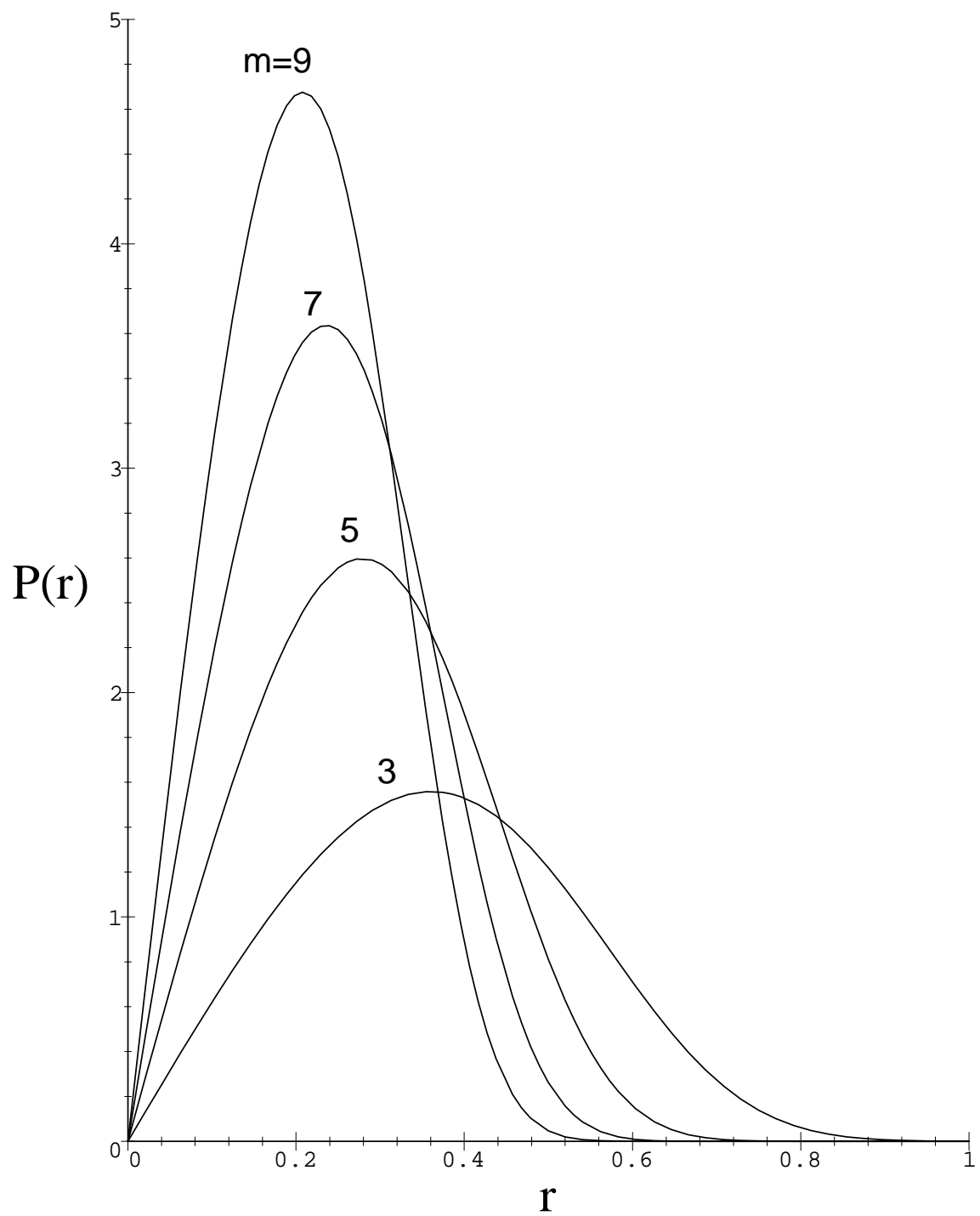
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FIG. 1: The scaled elastic free energy  $\beta F_{\alpha\beta}(4/m_{\alpha\beta}^2)$ .

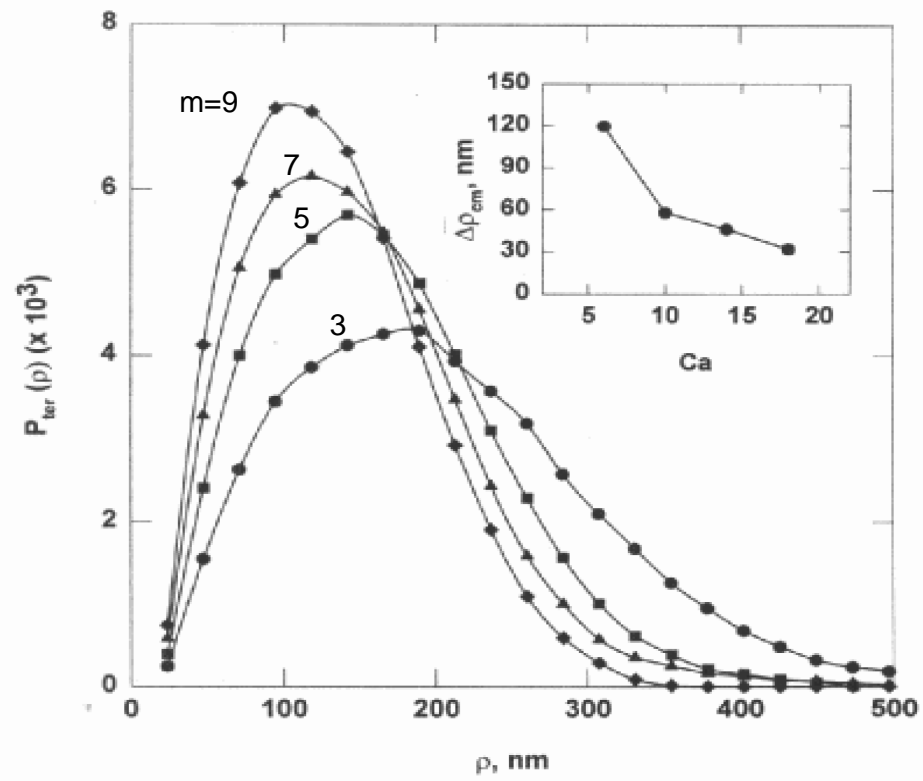
FIG. 2: Distribution functions for the distance (in the projection plane) between segments on separate rings for linking numbers  $m = 3, 5, 7, 9$ .

FIG.3 The experimetal result drawn from electron micrographs. It is not sure that these values correspond to thermal equilibrium. Thus a direct fit has been avoided.





$P(r)$



$r$